Randomized controlled crossover study of the effect of a highly β-glucan–enriched barley on cardiovascular disease risk factors in mildly hypercholesterolemic men[1–3]

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ABSTRACT

Background: Soluble-fiber β-glucan derived from oats can reduce cardiovascular disease (CVD) risk through reductions in total and LDL cholesterol. Barley-derived β-glucan may also improve serum cholesterol, but large quantities are required for clinical significance.

Objective: This trial investigated whether a β-glucan–enriched form of barley can favorably modify cholesterol and other markers of CVD and diabetes risk.

Design: Eighteen mildly hyperlipidemic ($\bar{x}$ ± SD: 4.0 ± 0.6 mmol LDL cholesterol/L) men with a mean (± SD) body mass index (in kg/m^2) of 27.4 ± 4.6 were randomly assigned in this single-blind, 2 × 4-wk trial to either the treatment arm [8.1–11.9 g β-glucan/d (scaled to body weight)] or the control arm (isoenergetic dose of 6.5–9.2 g glucose/d). After a washout period of 4 wk, dietary regimens were crossed over. The trial took place in a long-stay metabolic facility, and all foods were provided (38% of energy from fat). Fasted blood samples were collected on days 0, 1, 7, 14, 21, 28, and 29 in both study arms. An oral-glucose-tolerance test was carried out on days 0 and 29.

Results: There was no significant change (Δ) in total (Δ = −0.08 mmol/L, −1.3%), LDL (Δ = −0.15 mmol/L, −3.8%), or HDL (Δ = 0 mmol/L) cholesterol or in triacylglycerol (Δ = 0.18 mmol/L), fasting glucose (Δ = −0.05 mmol/L), or postprandial glucose when analyzed between treatments ($P > 0.05$; ANOVA).

Conclusion: The effect of β-glucan–enriched barley on lipid profile was highly variable between subjects, and there was no evidence of a clinically significant improvement in CVD risk across this group of mildly hyperlipidemic men. *Am J Clin Nutr* 2003;78:711–8.

KEY WORDS Serum cholesterol, soluble dietary fiber, β-glucan, barley, randomized controlled trial, hyperlipidemic men

INTRODUCTION

The protective effects of dietary fiber against cardiovascular disease (CVD), mediated through a reduction in serum lipids, was first reported >40 y ago by Keys (1); later research led to the dietary fiber hypothesis proposed by Burkitt (2) and Trowell (3) that a high intake of starchy carbohydrates and fiber is protective against cardiovascular disease. More than 140 intervention trials have since been carried out with the use of fiber supplements, fiber-enriched foods, and high-fiber whole foods (4–8) to investigate the relation between fiber intake and CVD risk factors. More than 80% of these trials showed a hypocholesterolemic effect of an increased intake of soluble fiber from guar gum and cereals such as oats and psyllium.

Within recent years, the US Food and Drug Administration has endorsed the relation between an increase in soluble fiber and a decrease in serum total cholesterol by ratifying health claims for oats (9) and for psyllium fiber (10). The active component in oats has been identified as the linear mixed-link (1→3)(1→4)-β-D-glucan (β-glucan) (11), which reduces serum total cholesterol by ≈5–10% (5) and which in oats is present at close to 4% (by wt) (12). Barley contains 5–10% (by wt) β-glucan (12) and so may be expected to have similar cholesterol-lowering effects, yet there are few published trials on barley cereal. The trials carried out used a variety of barley flour, bran, flakes, and brewer's spent yeast as the source of β-glucan, and most (13–20) although not all (21) showed barley β-glucan to be hypocholesterolemic. In addition, animal (22, 23), epidemiologic (24, 25), and human intervention (26–28) studies have shown that a diet high in soluble fiber may also improve glucose and insulin control and hence reduce the risk of type 2 diabetes (29–31). The high viscosity of β-glucan may be particularly effective at reducing postprandial glycemia (30), and several trials using oat or barley products (27, 29–37) reported significant reductions in glycemic response.

Barley, unlike oats, is not a readily accepted food source, and it is unlikely that sufficient amounts could be incorporated into

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the diet to achieve the recommended intake of 3 g β-glucan/d (9) without enrichment of the product. Viable options for dietary inclusion must be encapsulation or high enrichment in a manner similar to that used to incorporate oat gum into commercial food products. No trials of highly β-glucan–enriched barley were previously conducted. The aim of this study was to assess whether a highly β-glucan–enriched barley (75% by wt) would result in a clinically significant improvement in CVD risk in a group of men with mild hypercholesterolemia.

SUBJECTS AND METHODS

Subjects

Eighteen male volunteers were recruited into the study by means of an advertisement for interested participants. Subjects aged 18–65 y were recruited on the basis of a mildly elevated concentration of LDL cholesterol (> 3.5 mmol/L), no current drug treatment for hyperlipidemia, and no history of CVD. No subjects were currently being treated for hypertension, overweight or obesity, metabolic disorders including diabetes mellitus, or depression. All had normal liver and and thyroid functions. All volunteer subjects provided written informed consent. Ethical approval for this study was obtained from the University of Auckland and the Auckland North Health Authority ethics committees.

Protocol

This study was a randomized crossover intervention trial in which participants were blinded to treatment. Subjects were required to be resident at the University of Auckland Human Nutrition & Metabolic Unit throughout both dietary periods, and compliance was ensured by the provision of all foods and beverages during the intervention. Entry into the barley β-glucan treatment arm or the control arm of the trial was by random assignment using stratification to ensure that each arm was balanced. Each of the 2 intervention periods was 4 wk long, and they were separated by a minimum washout period of 4 wk, during which all volunteers returned home and resumed their normal diet. After the washout period, subjects crossed over to the other arm of the study. Blood and urine samples were routinely collected throughout the intervention. Blood samples were collected from fasted subjects by venipuncture on the morning of days 0 and 1 (preintervention baseline) and days 7, 14, 21, 28, and 29. An oral-glucose-tolerance test was also carried out on days 0 and 29 according to standard World Health Organization protocols (38). A liquid glucose polymer (Polycal; Nutricia-Bornem, Antwerp, Belgium), equivalent to 75 g anhydrous glucose and accepted for use by the World Health Organization, was used for the standard glucose load. 24-h urine samples to assess dietary compliance by nitrogen balance were collected on days 10 and 20 on both arms of the intervention. Body weight was measured daily while subjects were fasted and after voiding of the bladder. Blood samples were analyzed for total, LDL, and HDL cholesterol; triacylglycerol; fasting plasma glucose; and postprandial plasma glucose.

Treatment

The barley β-glucan fed in this trial was given as a highly enriched barley fiber product, a gelling form of β-glucan (Glucagel; Graceline Ltd, Christchurch, New Zealand), produced from high β-glucan content barley that was milled and sieved to separate the starch and cell-wall material. A 2-step extraction process was carried out to produce the β-glucan–enriched product: *1*) water extraction at 50–60 °C and *2*) a freeze-and-thaw extraction from which the β-glucan was recovered. The final product was a (1→3)(1→4)-β-D-glucan (β-glucan), comprising 75% (by wt) β-glucan that was insoluble in cold water and that formed a weak gelling agent. Active treatment comprised a daily supplement of 0.67 g barley β-glucan/MJ of the total diet and control comprised an isoenergetic amount of monosaccharide glucose of 0.54 g/MJ of the total diet. The dose of both treatment and control was scaled to body size for all subjects. The β-glucan treatment and glucose control supplements were incorporated into snacks and meals consumed throughout the day; cooked into bread, waffles, and muffins for breakfast; cooked into bread for sandwiches at lunch; cooked into savory dishes such as spaghetti Bolognese and chicken curry and into desserts for evening meals; and cooked into cakes and cookies for between-meal snacks.

Background diet

The background diet was designed to be identical on both arms of the intervention to ensure that the only nutrient that differed between treatments was β-glucan. The diet was controlled for macronutrient and micronutrient composition through careful measurement of all food ingredients, each of which was weighed to the nearest gram during diet preparation. The energy and macronutrient contents of the diet were calculated with the use of the dietary program, FOODWORKS (version 2.05; Xyris Software, Brisbane, Australia), which included data from the New Zealand Food Composition Database, to be 38% of energy from fat, 13% of energy from protein, and 49% of energy from carbohydrate, and this content was typical of a Westernized diet. Duplicate samples of the entire 7-d diet rotation were collected from each of 4 randomly chosen subjects on one occasion during the β-glucan–enriched barley treatment and on one occasion during the control treatment. Each of the 7-d samples was individually homogenized, and an alkquot was frozen for later chemical analysis. The content of dietary fiber was verified by direct chemical analyses from these duplicate diets by using the methods of Englyst et al (39).

Subjects were fed to energy balance, based on a multiple of predicted basal metabolic rate (40), and diets were reviewed by members of the research team on a daily basis to ensure that a constant body weight was maintained during each intervention period. A combination of change in body weight, reported activity, and hunger levels was used to assess total daily energy requirements. A 7-d dietary rotation was used so that every week the entire diet was repeated. Subjects were provided with breakfast, lunch, dinner, and between-meal snacks. Breakfast and dinner were eaten under supervision at the Nutrition Unit, and lunch and snacks were packed and participants were able to take them to college or their place of work as they chose. Decaffeinated, sugar-free sodas and decaffeinated, sugar-free tea and coffee were freely available. Subjects were asked to eat all of the foods provided for the trial and no others. Alcohol was prohibited throughout the intervention. The subjects were self-selected and well motivated. Independent dietary compliance was assessed from 24-h urinary nitrogen balance data, in which urinary losses of nitrogen were directly compared with dietary protein intake (where g protein = 6.25 × g

TABLE 1
Clinical characteristics at screening of 18 men who completed both arms of the crossover intervention[1]

Clinical variable	
Age (y)	38.8 ± 10.1
Body weight (kg)	86.3 ± 15.8
BMI (kg/m^2)	27.4 ± 4.6
Waist (cm)	96.1 ± 11.7
SBP (mm Hg)	128 ± 12.0
DBP (mm Hg)	86 ± 9.9
Total cholesterol (mmol/L)	5.9 ± 0.7
LDL cholesterol (mmol/L)	4.2 ± 0.7
HDL cholesterol (mmol/L)	0.9 ± 0.2
Triacylglycerol (mmol/L)	1.8 ± 0.9
Plasma glucose (mmol/L)	5.3 ± 0.5

[1] $\bar{x}$ ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure. All variables measured in subjects in the fasted state.

nitrogen). Para-aminobenzoic acid supplementation was used in an attempt to verify complete 24-h urine collections, according to the method of Bingham (41).

Statistical analysis

Body weight and metabolic outcomes (total, LDL, and HDL cholesterol; total:HDL; triacylglycerol; and glucose) were analyzed by using linear mixed-models analysis of variance (PROC MIXED, version 8.0; SAS Institute Inc, Cary, NC) and corrected for autocorrelation of errors over time. The dietary treatment, the arm of the trial (stratum), the intervention period, and the study day within period were explicitly modeled as fixed factors, as was the treatment × day interaction that addressed whether the trajectory over time during the intervention period differed between treatments (diet × time). Subjects within strata were treated as random, as were their interactions with day and intervention period. Repeat baseline measures before intervention (days 0 and 1) were combined into a single mean value to reduce variability at baseline. Repeat data collected at the end of the intervention (days 28 and 29) were not combined. Variable intervals between blood collections were also included in the analyses so that the unequal numbers of days between measurements were modeled as an autoregressive order 1 process with constant day-to-day correlation. Dietary composition measured by chemical analysis was analyzed between treatments by using paired Student's *t* test. All biochemical assays were analyzed in triplicate, and the values are presented as means ± SEMs. Statistical significance was based on 95% CIs ($P < 0.05$).

RESULTS

Eighteen male subjects, recruited on the basis of mildly elevated LDL cholesterol, entered the trial and completed both arms of the intervention (**Table 1**). There were no subjects who withdrew or who were excluded for noncompliance. The subjects were aged 26–61 y, and they tended to be overweight [mean body mass index (in kg/m^2): 27.4 ± 4.6; range: 22–39]. At baseline, mean total and LDL cholesterol concentrations were 5.8 ± 0.8 and 4.0 ± 0.6 mmol/L, respectively, both of which were above the ideal range for healthy men. HDL cholesterol, triacylglycerol, and glucose concentrations were all within the normal range, and there was no evidence of significant hypertension.

The average dose of barley β-glucan added to the diet on a daily basis during the period of active treatment was 9.9 ± 0.9 g/d (range: 8.1–11.9 g/d; **Table 2**). This dose was matched isoenergetically with an average dose of 7.7 ± 0.8 g glucose/d (range: 6.5–9.2) during the control arm. When measured by direct chemical analyses from the duplicate diets collected throughout the trial, the total fiber content of the diet, including the barley β-glucan supplement, was estimated to be 28.7 ± 2.6 g/d in the control period and 35.8 ± 4.8 g/d in the β-glucan period ($P < 0.001$; **Table 3**), the mean increase was 7.1 g/d. Soluble fiber was shown to increase significantly from 14.0 ± 1.1 to 20.7 ± 4.8 g/d between the control and β-glucan arms of the trial ($P < 0.001$). This was an estimated increase of 6.7 g/d, which showed that the analytic methods used for fiber analyses did not detect all of the β-glucan added into the diet, possibly because of poor solubility during 80% ethanol precipitation if the β-glucan were depolymerized, but they were somewhat informative. Insoluble fiber was kept almost constant between diets, at an average of 15.2 ± 1.9 and 15.8 ± 3.4 g/d on the control and β-glucan arms of the trial, respectively. Body weight was kept stable throughout both arms of the intervention through manipulation of energy intake on a daily basis in response both to changes in body weight and to the reported hunger of the subjects. There was no significant difference between body weight at baseline or weight change throughout the 4 wk intervention periods between the 2 treatments ($P > 0.05$). When subjects were on the

TABLE 2
Composition of the barley β-glucan and glucose control treatments

Composition	β-Glucan treatment		Glucose control treatment	
	Per 100 g	Amount[1]	Per 100 g	Amount[2]
Enriched barley fiber product (g)[3]	100	13.1	0	0
Soluble-fiber β-glucan (g)[4]	75	9.9 ± 0.9[5]	0	0
Total energy (kJ)	957	125 ± 13	1570	125 ± 13
Protein (g)	13	1.7 ± 0.2	0	0
Fat (g)	0	0	0	0
Total sugars (g)	7	0.9 ± 0.1	100	7.7 ± 0.8
Water (g)	5	0.7 ± 0.07	0	0

[1] Given to subjects; average dietary intake of 14.7 MJ/d.
[2] Given to subjects; average dietary intake of 14.4 MJ/d.
[3] 75% soluble-fiber β-glucan.
[4] Metabolizable energy content of soluble-fiber β-glucan based on 8.4 kJ/g (42).
[5] $\bar{x}$ ± SD.

TABLE 3
Composition of the diet during the treatment (barley β-glucan supplement) and control (glucose) arms of the trial as calculated and as measured by direct chemical analyses of a 7-d duplicate diet collected during the trial from a subset of 4 subjects[1]

	β-Glucan treatment	Glucose control treatment	Difference
EI, calculated (MJ/d)[2]	14.7 ± 0.1[3]	14.4 ± 0.1	0.3
Protein (% of energy)			
Calculated[2]	13	13	0
Measured[4]	12.1 ± 0.4	12.3 ± 0.5	−0.2
Carbohydrate (% of energy)			
Calculated[2]	49	49	0
Measured[4]	53.9 ± 2.3	54.5 ± 1.0	−0.6
Soluble fiber (g/d)			
Calculated[2]	NA	NA	9.9[5]
Measured[4,6]	20.7 ± 4.8[6]	14.0 ± 1.1	6.7[7]
Insoluble fiber (g/d)			
Calculated[2]	NA[5]	NA[5]	0[5]
Measured[4,6]	15.8 ± 3.4[6]	15.2 ± 1.9	0.6
Total fiber (g/d)			
Calculated[2]	37.0	27.0	10.0
Measured[4,6]	35.8 ± 4.8[6]	28.7 ± 2.6	7.1[7]
Fat (% of energy)			
Calculated[2]	38	38	0
Measured[4]	34.0 ± 2.0	33.2 ± 1.4	0.8
SFA, measured (% of energy)[4]	15.2 ± 2.4	14.0 ± 0.9	1.2
MUFA, measured (% of energy)[4]	12.5 ± 0.9	12.3 ± 0.3	0.2
PUFA, measured (% of energy)[4]	6.3 ± 1.7	6.9 ± 0.4	−0.6
Cholesterol, measured (mg/d)[4]	236 ± 43	237 ± 47	−1

[1] EI, energy intake; NA, not available; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.
[2] Calculated from FOODWORKS.
[3] $\bar{x}$ ± SD.
[4] Chemical analyses of duplicate diets for 4 subjects, adjusted for an average intake of 14.7 MJ/d on the treatment arm and 14.4 MJ/d on the control arm.
[5] Represents added fiber.
[6] Measured soluble-fiber and total-fiber contents of the diet were significantly higher during β-glucan supplementation (paired t test), $P < 0.001$.
[7] Calculated by the method of Englyst et al (39).

barley β-glucan treatment, mean body weight was 86.5 ± 3.6 and 86.8 ± 3.6 kg at baseline and at the end of the intervention, respectively; when subjects were on the control arm, mean body weight was 87.0 ± 3.5 and 87.1 ± 3.5 kg at baseline and at the end of the intervention, respectively. No subject lost or gained > 1 kg body wt during the intervention periods.

Blood samples were collected on 14 occasions from each of the 18 subjects during the 4-wk active and control treatment arms. There was no significant change in lipid profile between baseline and the end of the intervention on either the active treatment (time, $P > 0.05$; **Figure 1**) or control diet (time, $P > 0.05$), nor were there significant between-treatment effects of diet over time on total, LDL, and HDL cholesterol concentrations; total:HDL (diet × time, $P > 0.05$; Figure 1); or triacylglycerol (diet × time, $P > 0.05$; **Figure 2**). Total cholesterol changed by −0.09 and −0.02 mmol/L between baseline (days 0 and 1 combined) and the end of the intervention (day 29) on β-glucan and control diet, respectively. This represented a between-treatment differential of 1.3%. LDL cholesterol changed by −0.21 and −0.05 mmol/L between baseline and the end of the intervention on the β-glucan and the control diet, respectively. This represented a between-treatment differential of 3.8%. Throughout the barley β-glucan treatment, total and LDL cholesterol concentrations were consistently lower than when subjects were on the control arm, and there were also pronounced week-to-week fluctuations in circulating lipids during the active treatment. Both between-subject (0.68 mmol/L, 11.6%) and within-subject (0.36 mmol/L, 6.1%) variability in total cholesterol was high. When individual time points were analyzed within the linear mixed model as daily pairwise comparisons, there was no significant between-treatment effect of diet at day 7 or day 14 for any of the measured lipids—total, LDL, and HDL cholesterol concentrations; total:HDL; or triacylglycerol ($P > 0.05$). There was a significant between-treatment effect at day 21 for total cholesterol ($P < 0.01$) but not for LDL or HDL cholesterol, total:HDL, or triacylglycerol ($P > 0.05$), and this effect on total cholesterol was no longer detectable on the days after day 21. The least-squares mean ± SEM differences for total cholesterol between the treatment and control (after correcting for the other factors) were 0.042 ± 0.126 on day 14, 0.367 ± 0.126 on day 21, 0.076 ± 0.126 on day 28, and 0.05 ± 0.126 on day 29, with 2 outlier data points on control diet responsible for the effect. Whereas these changes may indicate some activity of the soluble fiber with respect to cholesterol lowering, there was neither consistency of effect nor significant difference between treatments when they were analyzed over the entire 4-wk intervention ($P > 0.05$).

Fasting plasma glucose tended to decrease over 4 wk on both treatments, but these decreases were not significant by the end of either intervention period (time, $P > 0.05$). There were no significant between-treatment diet × time effects on circulating fasting plasma glucose (diet × time, $P > 0.05$; Figure 2). When given an oral-glucose-tolerance test challenge, average postprandial plasma glucose changed in a predictable manner on both treatments, increasing to a peak at 60 min (t_{60min}) and returning to near baseline by 120 min (t_{120min}; **Figure 3**). Nine subjects were sufficiently insulin sensitive that a rise in glucose at t_{60min} was prevented for ≥ 1 of the 4 tests. Two subjects had flat curves on all 4 occasions. With the use of fasting plasma glucose concentrations > 5.5 mmol/L as an indicator, 6 subjects were shown to be mildly glucose intolerant on ≥ 1 of the 4 tests. Three subjects showed impaired glucose tolerance on all 4 tests. No subjects were shown to be diabetic. When analyzed as a group, there were no significant changes in the area under the 120-min glucose curve or in the glucose concentration at t_{120min} between baseline and day 29 on either the β-glucan or control diets ($P > 0.05$), nor were there significant changes with analysis between treatments ($P > 0.05$).

DISCUSSION

This randomized crossover trial was unable to provide evidence of a significant improvement in CVD risk or type 2 diabetes risk in a group of mildly hypercholesterolemic, middle-aged men fed a highly enriched form of barley-derived β-glucan as part of a typical 38% fat diet. Total cholesterol decreased by only 1.3% and LDL cholesterol by 3.8% over the 4-wk intervention period, which indicated a very modest improvement, if any, in the risk profile.

The 10 g/d dose given to subjects in this trial was more than 3 times the intake recommended by the Food and Drug Administration for efficacious action of soluble-fiber β-glucan (9), an intake level established from > 40 intervention studies feeding oat products

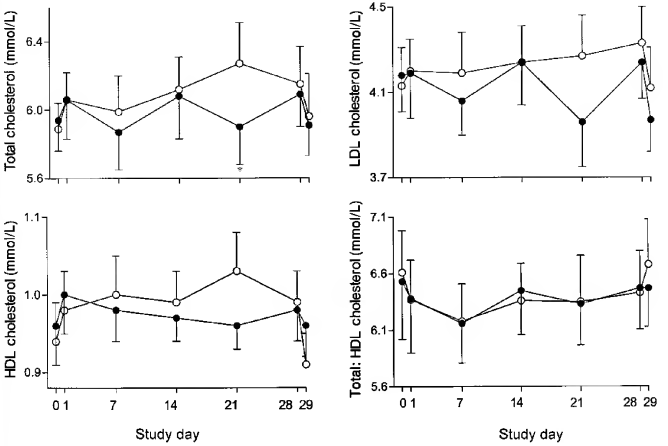

FIGURE 1. Mean (± SEM) total, LDL, and HDL cholesterol concentrations and the ratio of total to HDL cholesterol (total:HDL) during the 4-wk barley β-glucan (●) and control (○) treatments in 18 mildly hyperlipidemic men. There was no significant main effect of treatment (diet; ANOVA) for total ($P = 0.25$), LDL ($P = 0.37$), or HDL ($P = 0.34$) cholesterol or total:HDL ($P = 0.88$) or treatment × time interaction (diet × time; ANOVA) for total ($P = 0.24$), LDL ($P = 0.44$), or HDL ($P = 0.37$) cholesterol or total:HDL ($P = 0.92$). [2]Significantly different from control treatment, $P < 0.01$.

(43–52). Most of these trials, which fed oat cereal at doses of 3–145 g/d to both healthy and hyperlipidemic subjects for periods of 2–12 wk, showed 5–10% reductions in total cholesterol, which have been established as both statistically and clinically significant. The current study was based on this earlier body of evidence and powered to detect a 5% decrease in the primary outcome, total cholesterol. The variability in lipid profile in the group of hyperlipidemic subjects recruited into this trial was greater than predicted: 0.68 mmol/L (11.6%) between subjects and 0.36 mmol/L (6.1%) within subjects. This variability would have masked small improvements in circulating total cholesterol of < 5%. It is clear, however, that the β-glucan–enriched barley product used in this trial was unable to match the established efficacy of oat-soluble fiber when fed as oat fiber supplements, fiber-enriched ingredients, or mixed fibers (43–52) or of barley-soluble fiber given as mixed barley diet, barley bran flour, barley oil, brewer's spent grain, and barley β-glucans per se (13–15, 17–20, 53, 54). There have been far fewer trials investigating efficacy of barley β-glucan on CVD or type 2 diabetes risk factors or potential mechanisms of action (55), and no trials at all of β-glucan–enriched barley.

Debate exists as to the mechanism by which a soluble fiber such as β-glucan exerts its effect. Possible mechanisms include *1*) increased viscosity in the gastrointestinal tract (56–58), delay in cholesterol absorption, and increased conversion of cholesterol into bile acids (59, 60) through increased fecal bile acid excretion after binding, although there is little evidence that barley-soluble fiber binds bile acids (61); *2*) inhibition of cholesterol synthesis through short-chain fatty acid production (62, 63); and *3*) thickening of the unstirred layer of gut lumen, a consequence of an immune response leading to the secretion of materials including glycosaminoglycans, proteoglycans, and glycoproteins that collectively form a viscous mucin (G Coles, unpublished data, 2000). In comparison, insoluble fiber has little effect on serum cholesterol but improves the function of the large intestine by means of rapid intestinal transit time and gastric emptying (64, 65).

The relatively large amount of oat cereal that must be consumed (40 g oat bran or 60 g oatmeal) to achieve an intake of 3 g β-glucan led to the development of enriched forms of oat β-glucan. Seven trials investigated the efficacy of oat β-glucan at concentrations of 10–80% (44, 66–71). Only 4 of the trials reported significant cholesterol lowering (44, 67, 69, 71), which raised the issues of whether the process of enrichment may directly affect bioactivity and whether efficacy may be compromised during processing (72).

The study that we have reported is the first trial to investigate the efficacy of a β-glucan–enriched barley. Unlike oats, 3 g

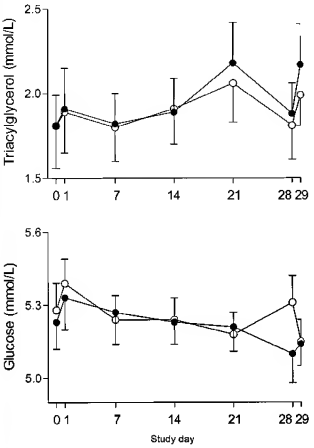

FIGURE 2. Mean (± SEM) triacylglycerol and fasting plasma glucose concentrations during the 4-wk barley β-glucan (●) and control (○) treatments in 18 mildly hyperlipidemic men. There was no significant main effect of treatment (diet; ANOVA) for triacylglycerol ($P = 0.61$) or fasting plasma glucose ($P = 0.32$) or treatment × time interaction (diet × time; ANOVA) for triacylglycerol ($P = 0.95$) or fasting plasma glucose ($P = 0.36$).

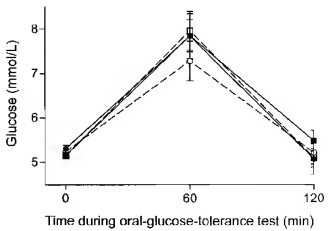

FIGURE 3. Mean (± SEM) change in venous glucose concentrations during an oral-glucose-tolerance test at baseline (●, solid line) and at the end of the 4-wk barley β-glucan treatment (■, solid line) and at baseline (○, dashed line) and at the end of the 4-wk control treatment (□, dashed line) in 18 mildly hyperlipidemic men. There were no significant differences between treatment and control arms (diet × time; ANOVA), $P = 0.34$.

barley β-glucan/d cannot be incorporated into a typical diet but must be highly enriched and consumed either as a supplementary food or nutraceutical product, such as psyllium fiber, or in a commercial food preparation, such as oat gum. In this trial, there was no evidence of a consistent improvement in serum lipids, despite the high dose of enriched β-glucan incorporated into the diet. The only significant between-treatment effect was that for total cholesterol at day 21 of intervention, and this single result may represent a type I error, exacerbated by 2 outliers. To investigate this further, within-treatment differences in total cholesterol between baseline and day 21 for active treatment were analyzed by pairwise t test, but they did not change significantly ($\Delta = 0.0944$, $t = 0.69$, $P = 0.5012$); hence the difference between treatment and control was not due to the effect of active treatment on cholesterol.

There are a number of possible reasons for reduced efficacy: *1*) unfavorable structural changes during commercial purification, such as depolymerization of the linear structure (72), that result in decreased molecular weight and reduced viscosity in the gastrointestinal tract; 2) mild extraction conditions (50–60 °C), which may not deactivate endogenous β-glucanases and may also lead to increased depolymerization (73, 74); *3*) cooking processes that in vitro digestion systems showed may reduce peak molecular weight (75), and *4*) freezing and storage, which were shown to reduce the extractability (75) but not the molecular weight (76) of oat β-glucan in the intestine. The quantity of β-glucan ingested accounts only in part for hypocholesterolemic effects, because the viscosity and molecular weight of soluble fiber in the gastrointestinal tract are critical (57, 58). A higher molecular weight may be associated with a higher viscosity and greater cholesterol reduction. A combination of these factors may help to explain the unexpectedly poor response in this trial.

There was no evidence of an improvement in glucose control, which may be unsurprising because no subjects were diabetic and few had impaired glucose tolerance. Many, although not all, of the animal and human trials that showed improvements in glucose control when a variety of soluble fibers were introduced into the diet were performed in prediabetic persons (22–37). Of the 10 trials that investigated responses to oats or barley (27, 29–37), all but one (33) reported significant improvement in glycemic response; however, only 5 of these trials tested the effect of β-glucan per se (30, 31, 34, 36, 37). Dietary fiber acts on glucose absorption and the rate of gastric emptying, which are determined largely by viscosity of fiber in solution (77). Reduction in postprandial glycemia has been attributed to the high viscosity of β-glucan (30).

In conclusion, this current trial—which investigated the efficacy of a highly β-glucan–enriched barley product—did not show clinically significant improvements in lipid or glucose control, and thus there was no evidence of improvement in CVD or type 2 diabetes risk in this group of young to middle-aged, mildly hypercholesterolemic men. Because β-glucan was previously shown to be highly efficacious at doses as low as 3 g/d, we suggest that this lack of effect may be, at least in part, a consequence of structural changes in β-glucan that result from the commercial processing of the barley into a highly enriched β-glucan product or from the

freezing, storage, or baking of the product during the intervention period. Highly enriched products of either barley or oat origin warrant further investigation to establish efficacy (78).

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GFK was the senior author, co-principal investigator, and trial coordinator. GJSC was the head of the research group, fundraiser, protocol designer, and clinician. TBM was the senior laboratory analyst (metabolic). BHM was the biostatistician. GC was responsible for the development of enriched barley β-glucan (Glucagel). JAM was the senior laboratory analyst (foods). SDP was the co-principal investigator, fundraiser, and director of the metabolic unit and had responsibility for protocol design and manuscript preparation. GDC is employed by Gracelinc, the manufacturer of Glucagel. None of the other authors had any conflicts of interest.

REFERENCES

1. Keys A, Anderson JT, Grande F. Diet-type (fats constant) and blood lipids in man. J Nutr 1960;70:257–66.
2. Burkitt DP, Walker ARP, Painter NS. Dietary fiber and disease. JAMA 1974;229:1068–74.
3. Trowell HC. Dietary fiber hypothesis of the etiology of diabetes mellitus. Diabetes 1975;24:762–5.
4. Anderson JW. Dietary fiber, lipids and atherosclerosis. Am J Cardiol 1987;60:17G–22G.
5. Ripsin CM, Keenan JM, Jacobs DR Jr, Elmer PJ, Welch RR, Van Horn L. Oat products and lipid lowering: a meta-analysis. JAMA 1992;267:3317–25.
6. Glore SR, Van Treeck D, Knehans AW, Guild M. Soluble fiber and serum lipids: a literature review. J Am Diet Assoc 1994;94:425–36.
7. Truswell AS. Dietary fiber and blood lipids. Curr Opin Lipidol 1995; 6:14–9.
8. Olson BH, Anderson SM, Becker MP. Psyllium-enriched cereals lower blood total cholesterol and LDL-cholesterol but not HDL-cholesterol in hyper-cholesterolemic adults: results of a meta-analysis. J Nutr 1997;127:1973–80.
9. Food and Drug Administration. Food labeling: health claims; oats and coronary heart disease; final rule. Rockville, MD: US Department of Health and Human Services, 1997. Internet: http://vm.cfsan.fda.gov/~lrd/fr970123.html (accessed 5 May 2002).
10. Food and Drug Administration. FDA allows foods containing psyllium to make health claim on reducing risk of heart disease. Rockville, MD: US Department of Health and Human Services, 1998. Internet: http://www.verity.fda.gov (accessed 5 May 2002).
11. Tietyen JL, Nevins DJ, Schneeman BO. Characterization of the hypocholesterolemic potential of oat bran. FASEB J 1990;4:A527 (abstr).
12. McIntosh GH, Newman RK, Newman CW. Barley foods and their influence on cholesterol metabolism. World Rev Nutr Diet 1995;77: 89–108.
13. Newman RK, Lewis SE, Newman CW, Boik RJ, Ramage RT. Hypocholesterolemic effect of barley foods on healthy men. Nutr Rep Int 1989;39:749–60.
14. Newman RK, Newman CW, Graham, H. The hypocholesterolemic function of barley β-glucans. Cereal Foods World 1989;34:883–6.
15. McIntosh GH, Whyte J, McArthur R, Nestel PJ. Barley and wheat foods: influence on plasma cholesterol concentrations in hypercholesterolemic men. Am J Clin Nutr 1991;53:1205–9.
16. Lupton JR, Robinson MC, Morin JL. Cholesterol-lowering effect of barley bran flour and oil. J Am Diet Assoc 1994;94:65–70.
17. Zhang J-X, Lundin E, Andersson H, et al. Brewer's spent grain, serum lipids and fecal sterol excretion in human subjects with ileostomies. J Nutr 1991;121:778–84.
18. Ikegami S, Tomita M, Honda S, et al. Effect of boiled barley-rice feeding in hypercholesterolemic and normolipemic subjects. Plant Foods Hum Nutr 1996;49:317–28.
19. Khalon TS, Chow FI. Hypocholesterolemic effects of oat, rice and barley dietary fiber and fractions. Cereal Foods World 1987;42:86–92.
20. Bourdon I, Yokoyama W, Davis P, et al. Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with β-glucan. Am J Clin Nutr 1999;69:55–63.
21. Narain JP, Shukla K, Bijlani RL, Kochhar KP, Karmarkar MG, Bala S. Metabolic responses to a four week barley supplement. Int J Food Sci Nutr 1992;43:41–6.
22. Gleason RE, Lauris V, Soeldner JS. Studies on experimental diabetes in the Wellesley hybrid mouse. III. Dietary effects and similar changes in a commercial Swiss-Hauschka strain. Diabetologia 1967; 3:175–8.
23. Gallagher DD, Olson JM, Larntz K. Dietary guar gum halts further renal enlargement in rats with established diabetes. J Nutr 1992;122: 2391–7.
24. Colditz GA, Manson JE, Stampfer MJ, Rossner B, Willett WC, Speizer FE. Diet and risk of clinical diabetes in women. Am J Clin Nutr 1992;55:1018–23.
25. Meyer KA, Kushi LH, Jacobs DR Jr, Slavin J, Sellers TA, Folsom AR. Carbohydrates, dietary fiber, and incident type 2 diabetes in older women. Am J Clin Nutr 2000;71:921–30.
26. Leclère CJ, Champ M, Boillot J, Guille G, Lecannu G, Molis C. Role of viscous guar gums in lowering the glycemic response after a solid meal. Am J Clin Nutr 1994;59:914–21.
27. Dubois C, Armand M, Senft M, et al. Chronic oat bran intake alters postprandial lipemia and lipoproteins in healthy adults. Am J Clin Nutr 1995;61:325–33.
28. Vuksan V, Sievenpiper JL, Owen R, Swilley JA, Spadafora P, Jenkins DJA. Beneficial effects of viscous dietary fiber from Konjac-mannan in subjects with insulin resistance syndrome. Diabetes Care 2000;23:9–23.
29. Holm J, Koellreutter B, Wursch P. Influence of sterilization, drying and oat bran enrichment of pasta on glucose and insulin responses in healthy subjects and on rate and extent of in vitro starch digestion. Eur J Clin Nutr 1992;46:629–40.
30. Wood PJ, Braaten JT, Scott FW, Riedel KD, Wolynetz MS, Collins MW. Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. Br J Nutr 1994;72:731–43.
31. Hallfrisch J, Scholfield DJ, Behall KM. Diets containing soluble oat extracts improve glucose and insulin responses of moderately hypercholesterolemic men and women. Am J Clin Nutr 1995;61: 379–84.
32. Bosello O, Ostuzzi R, Armellini F, Micciolo R, Scuro LA. Glucose tolerance and blood lipids in bran-fed patients with impaired glucose tolerance. Diabetes Care 1980;3:46–9.
33. Kestin M, Moss R, Clifton PM, Nestel PJ. Comparative effects of three cereal brans on plasma lipids, blood pressure, and glucose metabolism in mildly hypercholesterolemic men. Am J Clin Nutr 1990;52:661–6.
34. Braaten JT, Wood PJ, Scott FW, Riedel KD, Poste LM, Collins MW. Oat gum lowers glucose and insulin after and oral glucose load. Am J Clin Nutr 1991;53:1425–30.
35. Granfeldt YE, Hagander B, Björck I. Metabolic responses to starch in oat and wheat products. On the importance of structure, incomplete gelatinization or presence of viscous dietary fibre. Eur J Clin Nutr 1995;49:189–99.
36. Liljeberg HGM, Granfeldt YE, Björck IME. Products based on a high fiber barley genotype, but not on common barley or oats, lower postprandial glucose and insulin responses in healthy humans. J Nutr 1996;126:458–66.
37. Yokoyama WH, Hudson CA, Knuckles BE, et al. Effect of barley

beta-glucan in durham wheat pasta on human glycemic response. Cereal Chem 1997;74:293–6.
38. World Health Organization. Diabetes mellitus: report of a WHO study group. World Health Organ Tech Rep Ser 1985;727:1–113.
39. Englyst HN, Quigley ME, Hudson GJ. Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic, high performance liquid chromatographic or spectrophotometric measurement of constituent sugars. Analyst 1994;119:1497–509.
40. Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985;39:5–41.
41. Bingham SA. Urine nitrogen as an independent validatory measure of protein intake. Br J Nutr 1997;77:144–8.
42. Livesey G. Energy values of unavailable carbohydrate and diets: an enquiry and analysis. Am J Clin Nutr 1990;51:617–37.
43. Keenan JM, Wenz JB, Myers S, Ripsin C, Huang ZQ. Randomized, controlled, crossover trial of oat bran in hypercholesterolemic subjects. J Fam Pract 1991;33:600–8.
44. Braaten JT, Wood PJ, Scott FW, et al. Oat β-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. Eur J Clin Nutr 1994;48:465–74.
45. Uusitupa MIJ, Miettinen TA, Sarkkinen ES, Ruuskanen E, Kervinen K, Kesäniemi YA. Lathosterol and other non-cholesterol sterols during the treatment of hyper-cholesterolaemia with beta-glucan-rich oat bran. Eur J Clin Nutr 1997;51:607–11.
46. de Groot A, Luyken PR, Pikaar NA. Cholesterol-lowering effect of rolled oats. Lancet 1963;i:10:303–4 (letter).
47. Van Horn L, Emidy LA, Liu KA, et al. Serum lipid response to a fat-modified, oatmeal-enhanced diet. Prev Med 1988;17:377–86.
48. Anderson JW, Spencer DB, Hamilton CC, et al. Oat-bran cereal lowers serum total and LDL cholesterol in hypercholesterolemic men. Am J Clin Nutr 1990;52:495–9.
49. Marlett JA, Hosig KB, Vollendorf NW, Shinnick FL, Haack VS, Story JA. Mechanism of serum cholesterol reduction by oat bran. Hepatology 1994;20:1450–7.
50. Noakes M, Clifton PM, Nestel PJ, Le Leu R, McIntosh G. Effect of high amylose starch and oat bran on metabolic variables and bowel function in subjects with hypertrigyceridemia. Am J Clin Nutr 1996; 64:944–51.
51. Gerhardt AL, Gallo NB. Full-fat rice bran and oat bran similarly reduce hypercholesterolemia in humans. J Nutr 1998;128:865–9.
52. Jenkins DJA, Kendall CWC, Vuksan V. Viscous fibers, health claims, and strategies to reduce cardiovascular disease risk. Am J Clin Nutr 2000;71:401–2.
53. Klopfenstein CF, Hoseney RC. Cholesterol-lowering effect of β-glucans enriched bread. Nutr Rep Int 1987;36:1091 (abstr).
54. Roberts DC, Truswell AS, Bencke A, Dewar HM, Farmakalidis E. The cholesterol-lowering effect of a breakfast cereal containing psyllium fibre. Med J Aust 1994;161:660–4.
55. Lia A, Hallmans G, Sandberg A-S, Sundberg B, Åman P, Andersson H. Oat β-glucan increases bile acid excretion and a fiber-rich barley fraction increases cholesterol excretion in ileostomy subjects. Am J Clin Nutr 1995;62:1245–51.
56. Mälkki Y, Autio K. Oat bran concentrates: physical properties of beta-glucan and hypocholesterolemic effects. Cereal Chem 1992;69:647–53.
57. Gallagher DD, Hassel CA, Lee KJ, Gallagher CM. Viscosity and fermentability as attributes of dietary fiber responsible for the hypocholesterolemic effect in hamsters. J Nutr 1993 123: 244–52.
58. Marlett JA. Dietary fibre in cardiovascular disease. In: Cho SS, Dreher, eds. Handbook of dietary fibre. New York: Marcel Dekker, 2001:17–30.
59. Kritchevsky D, Story JA. Binding of bile salts in vitro by nonnutritive fiber. J Nutr 1974;104:458–62.
60. Bowles RK, Morgan KR, Furneaux RH, Coles GD. C-13 CP/MAS NMR study of the interaction of bile acids with barley beta-D-glucan. Carbohydr Polymers 1996;29:7–10.
61. Ebihara K, Schneeman BO. Interaction of bile acids, phospholipids, cholesterol and triglyceride with dietary fibers in the small intestine of rats. J Nutr 1989;119:1100–6.
62. Illman RJ, Topping DJ, McIntosh GH, et al. Hypercholesterolemic effects of dietary proprionate: studies in whole animals and perfused rat liver. Ann Nutr Metab 1988;32:95–107.
63. Cummings JC, Pomare EW, Branch WJ, Naylor CPE, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 1987;8:1221–7.
64. Jenkins DJ, Newton C, Leeds AR, Cummings JH. Effect of pectin, guar gum and wheat fibre on serum cholesterol. Lancet 1975;1: 1116–7.
65. Gallagher DD, Schneeman BO. Dietary fiber. In: Ziegler EE, Filer LJ, eds. Present knowledge in nutrition. Washington, DC: ILSI Press, 1996:87–96.
66. Lovegrove JA, Clohessy A, Milon H, Williams CM. Modest doses of β-glucan do not reduce the concentrations of potentially atherogenic lipoproteins. Am J Clin Nutr 2000;72:49–55.
67. Demark-Wahnefried W, Bowering J, Cohen PS. Reduced serum cholesterol with dietary change using fat-modified and oat bran supplemented diets. J Am Diet Assoc 1990;90:223–9.
68. Törrönen R, Kansanen L, Uusitupa M, Hanninen O, Myllymaki O, Harkonen H. Effects of an oatbran concentrate on serum lipids in free-living men with mild to moderate hypercholesterolemia. Eur J Clin Nutr 1992;46:621–7.
69. Uusitupa MI, Ruuskanen E, Makinen E, et al. A controlled study on the effect of beta-glucan enriched oat bran on serum lipids in hypercholesterolemic subjects: relation to apolipoprotein E phenotype. J Am Coll Nutr 1992;11:651–9.
70. Beer MU, Arrigoni E, Amadò R. Effects of oat gum on blood cholesterol levels in healthy young men. Eur J Clin Nutr 1995;49: 517–22.
71. Behall KM, Scholfield DJ, Hallfrisch J. Effect of beta-glucan level in oat fiber extracts on blood lipids in men and women. J Am Coll Nutr 1997;16:46–51.
72. Wursch P, Pi-Sunyer FX. The role of viscous soluble fiber in the metabolic control of diabetes—a review with special emphasis on cereals rich in beta-glucan. Diabetes Care 1997;20:1774–80.
73. Fastnaught C. Barley fibre. In: Cho SS, Dreher ML, eds. Handbook of dietary fibre. New York: Marcel Dekker, 2001:519–42.
74. McLeary BV. Measurement of dietary fibre components: the importance of enzyme purity, activity and specificity. In: McLeary BV, Prosky L, eds. Advanced dietary fibre technology. London: Blackwell Science Ltd, 2001:89–105.
75. Beer M, Wood P, Weisz J, Fillion N. Effect of cooking and storage on the amount and molecular weight of (1→3)(1Æ4)-beta-D-glucan extracted from oat products by an in vitro digestion system. Cereal Chem 1997;74:705–9.
76. Suortti T, Myllymaki O. Effect of some physical factors on molecular weight of beta-glucans. Am Assoc Cereal Chem. Charlotte, NC: American Association of Cereal Chemists, 2001:52.
77. Anderson JW, Chen W-JL. Plant fiber: carbohydrate and lipid metabolism. Am J Clin Nutr 1979;32:346–63.
78. Kerckhoffs DAJM, Hornstra G, Mensink RP. Cholesterol-lowering effect of β-glucan from oat bran in mildly hypercholesterolemic subjects may decrease when β-glucan is incorporated into bread and cookies. Am J Clin Nutr 2003;78:221–7.